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# TOXICOLOGICAL STUDIES ON THE MUSHROOMS CLITOCYBE ILLUDENS AND INOCYBE INFIDA

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With Plate 91

## Introduction

It had already become evident from our earlier observations that *Inocybe infida* must be considered a poisonous mushroom, both when judged by its effects on man after ingestion[1] and also from its action when injected into frogs.[2] Both the clinical data from the reported cases of poisoning and our own experimental results indicated a poison of the general type represented by the muscarin of the fatal *Amanita muscaria*. However, the usual symptoms were not produced in frogs receiving injections of the poison in *Inocybe infida*. As is the case with muscarin, the *Inocybe* poison acted more particularly upon the nervous system and seemed to be similar to the narcotic poison found by Ford[3] in the closely related *Inocybe infelix*. A more detailed study of the action of *Inocybe infida* and certain other fungi upon the *exposed heart* appeared to be desirable and in this paper we present the results of such experiments.

Increased interest in this work was stimulated by the very many fatalities, in the fall of 1911, which were caused by eating poisonous mushrooms. After the autumn rains in September of that year, the newspapers of the temperate zone of this continent and Europe contained many notices of death from this cause. In one period of ten days there were *twenty-two* such deaths in the vicinity of New York City. In scarcely a single fatal case was the identity of the fungi definitely established by a mycologist but usually the cause seemed to be either *Amanita phalloides* or *A. muscaria;* twin sisters of death, known and feared from antiquity. Many newspaper clippings from several states and countries indicate that the accidents often resulted from mistaking *Amanita phalloides* for *Agaricus campestris,* the common cultivated "mushroom," and *Amanita muscaria* for *A. caesaria,* much appreciated by a Caesar and later by somewhat reckless epicures. We have been concerned only with the less common and striking species such as *Inocybe* and *Clitocybe* but it is very desirable that all fungi having poisonous properties should be so designated and always treated with caution. With the exception of the pioneer work of Ford very little chemical and toxicological study has been given to American mushrooms.

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[1] Murrill. A New Poisonous Mushroom. Mycologia 1: 211. 1909.

[2] Clark and Kantor. Toxicological Experiments With Some of the Higher Fungi. Mycologia 3: 175–88. 1911.

[3] Ford. Distribution of Haemolysins, Agglutinins and Poisons in Fungi, etc. Jour. Pharmacol. and Exp. Therapeutics 2: 285–318. 1911.

## PREVIOUS TOXICOLOGICAL STUDIES ON CLITOCYBE AND INOCYBE

These two groups of fungi had not usually been regarded with suspicion as to their poisonous nature until various observers began to report unpleasant results following their consumption. Recently, specific charges of being poisonous have been brought against *Inocybe infida* by Murrill[1] and ourselves,[2] against *Inocybe infelix* by Ford,[3] against *Clitocybe dealbata sudorifica* by Ford and Sherrick,[4] and also against *Inocybe decipiens* by the same observers.[5] In *Clitocybe illudens* Ford[3] found a poison that was fatal to guinea-pigs but not to rabbits. As an edible fungus this plant has never been popular, possibly because its phosphorescent glow at night is not reassuring. Injection experiments with frogs showed that extracts of *Clitocybe multiceps* were harmless, as was expected. In the *Inocybe infida* we separated, by muscarin isolation methods, a water-soluble substance which, after injection into frogs, caused a prolonged state of paralysis often followed by complete recovery in a day or two. The aqueous extracts of *Inocybe infelix* prepared by Ford exerted

[4] Ford and Sherrick. On the Properties of Several Species of the Polyporaceae and of a New Clitocybe, etc. Jour. Pharmacol. and Exp. Therapeutics **2**: 549–58. 1911.

[5] Ford and Sherrick. Further Observations on Fungi, etc. Jour. Pharmacol. and Exp. Therapeutics **4**: 321–32. 1913.

a powerful narcotic effect on the nervous system of rabbits and guinea-pigs, described as a profound depression lasting for hours, sometimes with complete recovery. It is evident that these two *Inocybes* contain poisonous material acting as a strong narcotic agent but not necessarily causing death. Muscarin itself does not produce the symptoms already described and the mixed poisons of *Amanita muscaria* give still another series of effects upon the organism. It is believed by many investigators that muscarin *alone* is not responsible for all of the fatal effects following ingestion of *Amanita muscaria.* Both in clinical work and in animal experiments one finds that although atropin is an antidote for *pure* muscarin it does not wholly neutralize the toxic action of *A. muscaria.* Some of our experiments indicate that muscarin may be present in other fungi like *Inocybe* and *Clitocybe* whose effects are neutralized by atropin while this was not wholly true of our extracts of *Amanita muscaria.*

## EXPERIMENTAL PART

*Description of Botanical Material.*—All of the fungi used by us were identified and labelled for us by Dr. Murrill. Our large lot of *Clitocybe illudens* was collected near the New York Botanical Garden in the summer of 1911. The *Inocybe infida* material was also collected during the summer of 1911 on the lawns of the New York Botanical Garden. We used *Amanita muscaria* that came from Rochdale, Massachusetts, in August, 1911. Dried plants of species of *Clitocybe* and *Amanita* are large and easily handled while those of *Inocybe* are very small after desiccation and usually five or more did not weigh more than one gram. The dry fungi were ground repeatedly in a coffee-mill until a very fine powder was obtained. This powder was weighed and extracted as described below.

*Description of Chemical Methods Employed.*—The powdered fungi were extracted twice for twenty-four hour periods, at room temperature, with ten times their weight of 95 per cent. alcohol. The alcoholic extracts were carefully evaporated to a thick syrup which was in turn extracted with several 10 c.c. portions of alcohol. These repeated alcohol extractions and subsequent evaporations were continued until practically all of the fats, sugars and gums had been eliminated. A final extract of the last thick syrup was made with absolute alcohol and, after this had been evaporated, the syrupy residue was mixed with powdered glass and dried in a vacuum desiccator until a yellowish, friable mass resulted. This powder was extracted with a small volume of absolute alcohol, the latter was removed from the extract by evaporation, and the small amount of yellowish waxy residue was taken up in physiological saline solution after which the traces of insoluble matter were removed by filtration. Unless otherwise noted this solution of the toxic material was used for injection or for experiments with the exposed hearts. In order to separate and purify any toxic alkaloidal material we took up the last residue from absolute alcohol with water, added a little dilute sulphuric acid and finally a solution of potassio-mercuric iodid until no more of the yellow granular precipitate formed. The details of the chemical treatment of this substance, precipitated by the potassio-mercuric iodid, may be found in our earlier paper.[6] At the final stage of this treatment the small amount of poisonous water-soluble material was dissolved in physiological salt solution and then used for the toxicological experiments. This process of alcohol extraction and alkaloidal purification was originally planned to isolate muscarin from *Amanita muscaria;* we found it to be adequate for this purpose. We also found that this same process would separate and concentrate the toxic material in *Inocybe infida* and *Clitocybe illudens.* Chemically, then, the poison in the latter forms seemed to belong to the muscarin type but that question could finally be decided only by experiments on animals.

## TOXICOLOGICAL EXPERIMENTS WITH EXPOSED HEARTS

The hearts of medium-sized pithed frogs were exposed and connected in the usual way for the preparation of graphic records on a kymograph drum. In several cases the exposed hearts of turtles were used but with no appreciable difference in the type of records obtained. Solutions of the toxic material in physiological salt solution were always used on the hearts and in no case was the effect of an extract tested until we first obtained a normal tracing from the heart. The exposed hearts were kept moist with physiological saline solution and all traces of a previous test solution were washed away before adding an atropin solution, etc. To test the neutralizing effects of atropin we used a 0.5 per cent. solution of the sulphate salt of this alkaloid. Many tracings from different mushroom preparations were obtained but we shall only describe briefly and illustrate a few of the typical ones.

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[6] Loc. cit.

### *Experiments with Inocybe infida Extracts before Alkaloidal Purification*

*Experiment 6.*—April 20, 1912. Weight of frog was 32 gm. After placing this solution on the heart the frequency and amplitude of the beats both decreased until a complete standstill was produced. This was overcome in a few seconds by flushing the heart with atropin solution.

*Experiment 10.*—May 22. The heart of a decerebrated turtle (weight 583 gm.) was exposed, treated with an *Inocybe* extract, and the effect studied. The extract in this case was much more concentrated than that used in Experiment 6 and with the turtle heart it caused a complete standstill in 5 seconds. This effect was neutralized by atropin in 10 seconds.

*Experiment 4.*—April 16. Weight of frog was 28 gm. The *Inocybe* extract used was the same as that in Experiment 6 and it showed the same characteristic action on this heart. See Plate 91, fig. 1.

### *Experiments with Inocybe infida Extracts after Alkaloidal Purification.*

Some of the mushroom extracts were purified by the chemical process described and when these extracts were tested on frogs they were found to have retained their toxic properties unchanged or, more often, to have had them augmented. This would indicate that the toxic material is precipitated and purified by methods used for alkaloids and muscarin.

*Experiment 24.*—June 6. The frog weighed 36 gm. This purified extract was very active in causing a complete standstill which was relieved in a few seconds by atropin. See Plate 91, fig. 2.

### *Experiments with the Ash of an Active Unpurified Inocybe Extract*

*Experiment 21.*—May 29. About 15 c.c. of the extract used in Exp. 10 were evaporated to dryness and carefully ashed at low red heat, the ash was dissolved in water and tested on the heart of a frog weighing 29 gm. This solution did not produce the slightest flutter in the normal tracing and so it is evident that the inorganic or ash constituents of the fungus are not responsible for its toxic action.

### *Experiments with Unpurified Extracts of Amanita muscaria*

*Experiment 9.*—May 22. The action of an *Amanita* extract was tested upon the heart of a frog weighing 33 gm. In this case a standstill of the heart was not caused although the usual retarding effect was noticed. This effect was partially neutralized by atropin.

*Experiment 11.*—May 23. Experiment 9 was repeated with a frog weighing 29 gm. The same slight muscarin effect appeared.

### *Experiments with Purified Extracts of Amanita muscaria*

*Experiment 26.*—June 3. The action of a purified extract of this *Amanita* on a frog (weight 31 gm.) was similar to that recorded in Experiments 9 and 11 and it did not prove to be nearly as toxic as extracts of *Inocybe infida* or *Clitocybe illudens.*

### *Experiments with Unpurified Extracts of Clitocybe illudens*

*Experiment 5.*—April 16. Frog weighed 35 gm. The heart was soon brought to a standstill but was started at once by atropin. The action was exactly the same as that of *Inocybe infida* but it did not take place as rapidly. See Plate 91, fig. 3.

*Experiment 14.*—May 23. Turtle weighed 633 gm. The turtle's isolated heart reacted to *Clitocybe* extract in a way difficult to distinguish from the response to the *Inocybe* extract.

### *Experiments with Purified Clitocybe illudens Extracts*

*Experiment 33.*—June 6. Frog weighed 28 gm. The action of this purified *Clitocybe* preparation was the same as that of the unpurified extracts.

### *Experiments with the Harmless Clitocybe multiceps Extracts*

*Experiment 20.*—May 29. As a control on our processes we tested the unpurified extract of this fungus on the heart of a frog (weight 33 gm.). It was found to be entirely without action. Evidently we had not been producing toxic effects through our procedures.

## TOXICOLOGICAL EXPERIMENTS ON FROGS

In order to study the effect of the various fungi upon animal organisms as a whole we made injections of the different preparations into the dorsal lymph-sacs of frogs. The extracts were portions of those that were used in the exposed heart experiments; their preparation has already been described. Typical experiments with the different mushroom extracts will be given in order to show the characteristic action of each species.

### *Experiments with Purified and Unpurified Extracts of Amanita muscaria*

*Experiment 30.* June 3, 1912. Frog 11 weighed 24 gm. Received an injection of 1 c.c. of *purified* extract at 5.47 P.M.

5.49 P.M. Partly paralyzed.
5.52 P.M. Wholly paralyzed.
5.56 P.M. Heart stopped.
5.58 P.M. Received injection of 1 c.c. atropin solution.
6.03 P.M. Heart begins to beat weakly and irregularly.
June 4, 9.15 A.M. Frog 11 found dead.

The results of parallel experiments with *unpurified Amanita* extracts differed in no way from those indicated above.

### *Experiments with Purified Extracts of Inocybe infida*

*Experiment 28.*—June 3. Frog 8 weighed 23 gm. Received injection of 1 c.c. of this extract at 3.46 P.M.

3.48 P.M. Partly paralyzed.

3.49 P.M. Wholly paralyzed.
3.51 P.M. Heart at a standstill.
3.53 P.M. Received injection of 1 c.c. atropin solution.
3.56 P.M. Heart begins to beat slowly and it gradually resumes its normal action.
5.45 P.M. Frog 8 appears normal.

While this frog was wholly paralyzed we bared the sciatic nerve and, upon stimulation with an induced current, we obtained a normal response, thus indicating that no curare effect was present. Other experiments with *Inocybe* material gave similar results. In some cases the frogs died before the next morning; and in others, recovery seemed to be complete.

### *Experiments with Purified and Unpurified Extracts of Clitocybe illudens*

*Experiment 13.* May 23. Frog 3 weighed 28 gm. Received injection of 1.5 c.c. of *unpurified* extract at 5.24 P.M.
5.28 P.M. Paralyzed.
5.33 P.M. Heart at standstill.
5.38 P.M. Tested as above indicated; there was no curare effect.

*Experiment 27.* May 27. Frog 9 weighed 36 gm. Received injection of 1.5 c.c. of *purified Clitocybe* extract at 12.25 P.M.
12.30 P.M. Lethargic.
12.40 P.M. Paralyzed; heart at standstill.
12.42 P.M. Received injection of 1 c.c. atropin solution.
12.50 P.M. Heart beats irregularly.
5.30 P.M. Frog 9 found dead.

## CONCLUSIONS

It is evident that both *Clitocybe illudens* and *Inocybe infida* contain material exerting a characteristic muscarin effect when tested upon the exposed hearts of frogs and turtles. Furthermore, this toxic action on the exposed heart is completely overcome by the application of atropin sulphate solutions; an action analogous to that with a *pure* muscarin preparation. For some reason, parallel experiments with extracts of *Amanita muscaria* extracts did not show the expected toxic effects nor were the latter wholly neutralized by atropin. This may be explained by assuming that this *Amanita* contains another toxic substance as well as muscarin. In fact, this theory has been advanced by other investigators to account for the results of their clinical observations and animal experiments. The lot of *Amanita muscaria* used in this work did not cause the acute muscarin symptoms which were expected from the experiments in our earlier paper. It is interesting to note that this last lot of the *Amanita* was collected in a different state and at a different time, thus giving an opportunity for both the seasonal and local variations in toxicity reported by others.

Injection experiments with *Clitocybe illudens* and *Inocybe infida* confirmed the results obtained with the isolated hearts. The toxicological evidence of all our experiments points toward muscarin as the poison in *Inocybe infida* and *Clitocybe illudens,* and this idea gains further confirmation from the fact that our methods of extraction and purification were those originally suggested for the isolation of muscarin and alkaloidal substances from fungi. Parallel experiments with the edible *Clitocybe multiceps* revealed no toxicity of any sort. The ash constituents of *Clitocybe illudens* are not responsible for its toxicity. It was necessary to test this point because it has been stated that salts of potassium produce a muscarin-like effect on the heart.[7] From our studies on *Clitocybe illudens* and *Inocybe infida* it is plain that these plants should not be eaten, since all of them contain material having a dangerous action upon the nervous system.

We wish to thank Prof. Wm. J. Gies and Prof. F. S. Lee, of the College of Physicians and Surgeons, for much helpful advice. Dr. W. A. Murrill suggested this study of poisonous fungi and our thanks are also due him for providing and identifying most of the material used.

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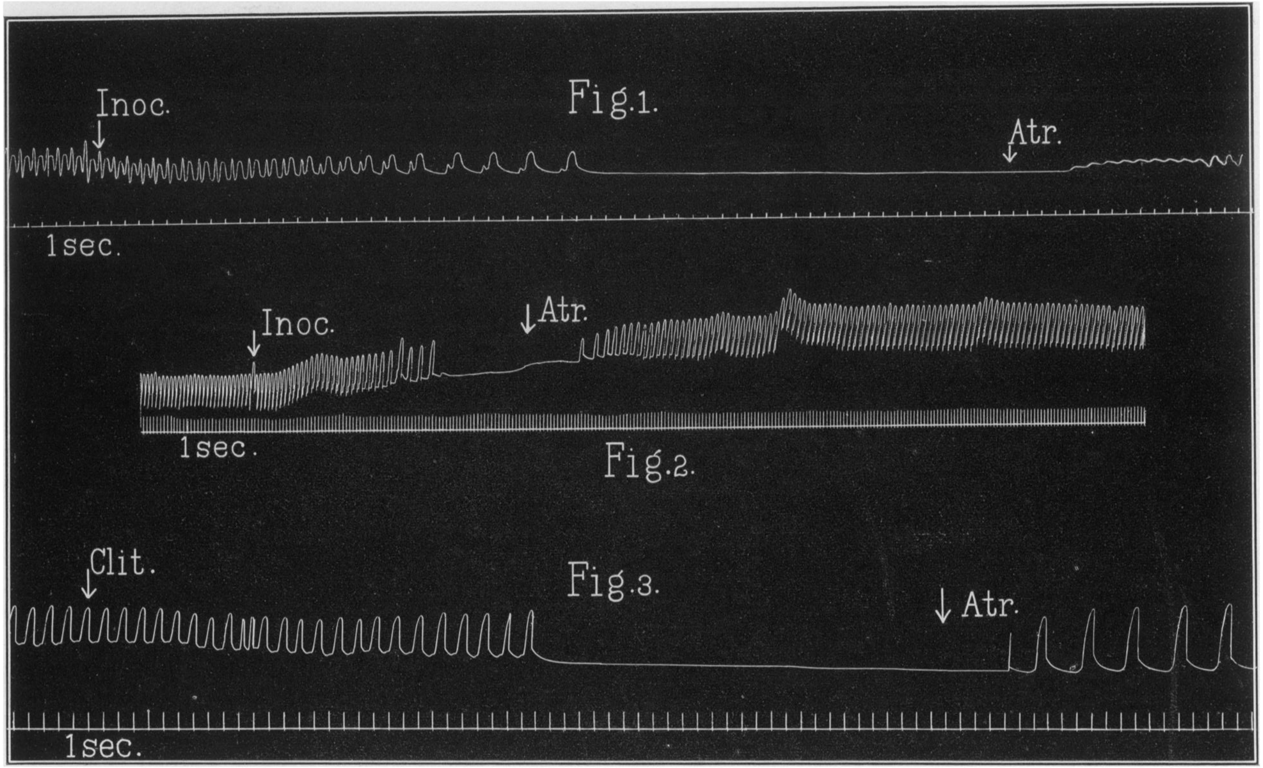
[7] Fühner. Nachweis und Bestimmung von Giften auf biologischen Wege. Abderhalden's Handbuch d. Biochem. Arbeitsmethoden, V, (I), p. 77. 1912.



Graphic records of effect of mushroom extracts on frog hearts. The arrows indicate time when the various mushroom extracts and atropin solution were applied to the exposed heart